

Protocol of the month

In this brand-new section published in association with Cold Spring Harbor Laboratory Press, Jaiswal and colleagues describe, step by step, a procedure to use quantum dots as labeling agents for fluorescence microscopy. Owing to their enhanced brightness, narrow emission spectra, and resistance to photodamage and metabolic degradation, quantum dots present advantages over organic fluorophores. This protocol shows how to conjugate quantum dots to biomolecules and specifically label proteins in fixed cells or on the surface of live cells. The versatility of the approach allows for simultaneous multicolor imaging of various cellular targets. **Protocol p73**

MicroRNA microarray

Understanding the function of microRNAs requires the ability to determine their expression level in relation with those of their potential targets. Hammond and colleagues have exploited the oligonucleotide microarray technology to quantify the expression level of large sets of microRNAs. They have optimized a method of RNA fluorescence labeling to overcome limitations imposed by the small size and structure of microRNAs. This procedure, coupled with the design of an oligonucleotide microarray based on known mouse and human microRNA sequences, allows for microRNA expression profiling from small amounts of starting cellular material and rapid assessment of microRNA involvement in a wide range of cellular and developmental processes. **Article p47**

Protein interaction sites tipped off

Solving the crystal structure of a protein is the most precise way to identify protein-protein interaction sites, but it is not always an easy task. Pfeffer and colleagues took a different approach to predict interaction sites in TIP47, a protein for which no crystal structure was available. By expressing the protein in bacteria harboring specific misincorporator tRNAs, the authors rapidly generated an ensemble of mutant proteins containing one randomly located cysteine residue per protein molecule. Monitoring cysteine reactivity in these mutants allowed for identification of surface positions and generation of a surface map, while affinity purification easily selected mutants with

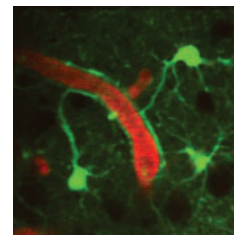
enhanced or decreased binding to a known partner. Comparison with the TIP47 crystal structure, solved by Hurley's group while the article was under review, validated the approach as a rapid and accurate way to predict interaction interfaces. **Article p55**

Finding a chromosome's center

Centromeric regions on higher eukaryotic chromosomes contain genes and functional elements embedded in highly methylated, repetitive DNA. The repetitive content of the centromeric DNA has turned the genetic and physical mapping of centromere regions into a laborious effort, which in the case of *Arabidopsis thaliana* took years to complete. By using a whole-genome fractionation technique, Daphne Preuss and her co-workers significantly reduced the time and effort needed to isolate centromeric DNA in *Arabidopsis*. They have also identified centromere regions in other, less well characterized plant species. This opens the interesting possibility that whole-genome fractionation could be used for higher organisms whose centromeres are as yet unmapped. **Article p67, News and Views p14**

Beginning to see the light: Astrocytes exposed *in vivo*

Signaling in the central nervous system depends critically on interplay between different cell types such as neuronal and glial cells. There are two prerequisites to visualizing the signaling of a particular cell type *in vivo*: adequate



imaging technologies and cell-specific staining. Two-photon microscopy has provided the necessary imaging tool, and now Nimmerjahn *et al.* have found a fluorescent dye, sulforhodamine 101 (SR101), that specifically stains astrocytes in rodent neocortex without labeling either neuronal cells, microglial cells or oligodendrocytes. SR101 allowed a morphological characterization of astrocytes, showing that they are closely associated with the neocortical microvasculature and may contribute to the regulation of local blood flow. To investigate astrocyte-specific calcium signaling in neocortex, the authors combined SR101 with a cell-permeant fluorescent calcium indicator. The differences they observed in signaling kinetics between neuronal and astroglial cells encourage studies addressing the interdependence of signaling in those cell types.

Article p31